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The Concept of the Mechanism of Oxygen Formation during Photosynthesis of Plants and Its Substantiation

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Molecular oxygen of the biosphere is formed during photosynthesis of plants, which takes place in the complicated permolecular structure of the oxygen-evolving complex (OEC) of chloroplast membranes as a result of oxidizing water molecules by using solar light energy. The absorption of light energy, its transformation into electrochemical energy of separated charges, and accumulation of oxidative equivalents in the water-oxidizing center (WC) on Mn cations performing water oxidation with O_2 formation are implemented in the complex. In order to decipher the mechanism of the functioning of the OEC, we have developed the method for chromatographic isolation of the complex from chloroplast membranes and pursued its comprehensive research. Based on the obtained data, we have ascertained the OEC to represent a dimer of pigment-lipoprotein complexes of photosystem 2 (PLPC PS-2) associated by the mirror symmetry rule on the basis of hydrophobic bonds. The dissociation of the OEC into monomeric complexes was observed under the action of heat (33-34^{o}C), solubilizing detergents, and heavy metals and resulted in the inhibition of the oxygen formation function. As a result of studying the structural transitions and regularities of the functioning of the OEC, we have first suggested the model of the molecular organization of the dimeric OEC and topographic location of its main proteins that has already been proved. The formation of the WC has been shown to be determined by the symmetry in the location of two unidentant ligands (Tyr_Z) coordinationally bound with Mn^{2+} cations in the structure of a hydrophobic boiler formed during the association of the monomeric PLPC PS-2. The oxygen formation mechanism has been developed, according to which the photochemical reactions taking place in turn in the left and right PLPC PS-2 in the process of the four-quantum cycle result in the removal of four electrons from two oppositely located Tyr_Z–liganded Mn cations. This determines the formation of a molecular two-anode reactor, in which photooxidized manganese cations enter into the reaction of photohydrolysis with water molecules and form a dihydroxide association [Mn^{4+}(OH)···(HO)Mn^{4+}] that determines the spontaneous electron density disproportionation between Mn cations and oxygen atoms of the hydroxyl groups of water molecules incorporated into the association. The synchronous and equal-rate but oppositely directed transfer of two pairs of electrons from the hydroxyl groups of water molecules to Mn^{4+} cations is the condition for the formation of molecular σ-π-orbitals of O_2. As a result, two Mn^{2+} cations are formed, molecular oxygen evolves, protons are released, and the initial state of the WC is regenerated. The quantum-chemical modeling has proved the suggested mechanism of molecular oxygen formation.

Keywords: photosynthesis, pigment-lipoprotein complex, photosystem 2, oxygen formation, quantum-chemical modeling

INTRODUCTION

Molecular oxygen formation during photosynthesis of plants is the key process of development of life on the Earth that is constantly in the center of attention of scientists (Joliot et al., 1969; Kok et al., 1970; Kutyurin, 1970; Blyumenfeld, 1977; Rutherford, 1989; Debus, 1992; Vermass at al., 1993; Shutilova, 1997 a, b; 1999; 2000; 2010; Hillier, 2001; Vrettos et al., 2001; Komissarov, 2002; Barber, 2003; Messinger, 2004; Yachadra, 2005; Dau and Haumann, 2007; Shutilova and Moiseev, 2010 a). The first signs of the origin of O_2 were shown to date back to the period of 2.5 billion years ago first in cyanobacteria as well as thermophilic and acidophilic algae and then, in the subsequent periods, in higher plants. The important achievement was to ascertain the identity between the isotopic composition of photosynthetic oxygen and oxygen of intracellular water and to get the evidences that water is the only and direct source of photosynthetic oxygen. These conclusions were first made in the works (Ruben et al., 1941; Vinogradov and Teis, 1941) and a whole series of research by these authors. Then, using the precision methods of isotopic analysis, the parameters of the isotopic composition of atmospheric oxygen were determined, and the constants of the isotopic exchange of oxygen atoms in plants and factors of its change were specified. This enabled the water origin

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of photosynthetic O_2 to be finally proved and permitted the possibility of its origin from any other oxygen-containing compound to be completely excluded (Kutyurin, 1970).

The research on the mechanism of photosynthetic water oxidation and oxygen formation showed its exclusive complexity. This process was clear to take place with the help of a photoexcited pigment and to correspond to the equation of four-electron oxidation; consequently, according to the second law of photochemistry, it required the absorption of four photons or four light quanta:

$$2H_2O + 4h\nu \rightarrow O_2 + 4H^+ + 4\bar{e}$$

The theoretical developments devoted to this problem showed that the complexity of the process was the implementation of the conditions for the origin of a bond between oxygen atoms of oxidized water molecules and prevention of the origin of highly active hydroxyl radicals rather than the performance of the photochemical reaction of the thermodynamically advantageous electron transfer from water to the molecule of a photoexcited pigment. The formation of highly active hydroxyl radicals would take place as a result of single-electron water oxidation in the process of the photochemical reaction. Undoubtedly, this is impermissible, since hydroxyl radicals provoke non-controlled chain reactions and destruction of surrounding macromolecules. It is also necessary to emphasize that another product of the reaction than can be formed during the recombination of hydroxyl radicals, such as hydrogen peroxide that is a biradical and highly active oxidant as well as hydroperoxide, can perform the destructive peroxide oxidation of, for example, pigments and non-saturated lipids forming chloroplast membranes. For this reason, when the mechanism of photosynthetic water oxidation was considered in theory, it was necessary to exclude the formation of products of radical nature (Blyumenfeld, 1977).

The structural organization of the water-oxidizing center that fixes the spatial location of initial water molecules and ensures the process of accumulation of the oxidative potential needed for the oxygen formation reaction was shown (Shutilova, 1997 a, b; 1999; 2000; Shutilova and Moiseev, 2010 a) to be the major factor determining the oxygen formation process during photosynthesis. The principles of organization of this molecular construction were formulated in (Blyumenfeld, 1977). They included the substantiation of the need for the existence of a screened reactionary space forming the water oxidation system in plant chloroplasts. This corresponded to the experimental data on the four-quantum periodicity of O_2 yield in response to a light flash (Joliot et al., 1969; Kok et al., 1970) that proved the need for the intermediate stabilization of oxidative equivalents. The concept was suggested that four photoreactions took place sequentially being accompanied by the removal of four electrons and transition of the system into more oxidized states $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_4$ (Kok et al., 1970). Only after the S_4 state is formed, water oxidation takes place that results in oxygen formation and proton release. As this occurs, the system returns to the initial S_0 state, and the four-quantum cycle of oxygen formation restarts. The Mn^{2+}, Mn^{3+}, Mn^{4+} cations, on which a positive charge is accumulated and the potential sufficient for oxidation of water molecules is formed, were ascertained to take part in these processes (Rutherford, 1989; Debus, 1992; Vermaas et al., 1993; Vrettos et al., 2001; Dau and Haumann, 2007; Yachandra, 2005). The synchronous removal of four electrons from two water molecules was grounded theoretically to be the most energetically advantageous (Shutilova, 1997 a, b; 2000; Shutilova and Moiseev, 2010 a). However, at present there is no common opinion about the mechanism of participation of manganese cations in the water oxidation process and no common opinion about their quantitative ratio and the method of their stabilization. It is also important that attention of researchers was not focused on revealing the structural factors of the formation of the water-oxidizing center that determine the kinetic and thermodynamic conditions of the processes of water oxidation and oxygen formation, escaping the stage where radicals originate. A series of analytical reviews consider the pursued research on ascertainment of the molecular organization of the OEC and approaches to understanding the mechanism of its functioning (Shutilova, 1999; 1997 a; 2010; Hillier, 2001; Komissarov, 2002; Barber, 2003; Messinger, 2004).

To decipher the mechanism of water oxidation and oxygen formation, it was necessary to study the structure of the isolated photosystem 2 submembrane complex that is responsible for the oxygen formation process in plant chloroplasts. With this purpose, we developed a method for isolating the oxygen-evolving complex from chloroplast membranes that permits its structure and functional activity to be retained (Shutilova and Kutyurin, 1976). During this research, a new methodology for solubilization of chloroplast membranes was created, and methods were developed for separating and purifying the submembrane pigment-lipoprotein complexes using ion-exchange chromatography. The division of the submembrane complexes into three types (the pigment-lipoprotein complex of photosystem 1 (PLPC PS-1), pigment-lipoprotein complex of

photosystem 2 (PLPC PS-2), and accessory light-harvesting complex (LH PLPC) (Shutilova and Kutyurin, 1976) was first achieved on their basis. The highly purified submembrane fragments of PS-2 (Shutilova et al., 1975) and reaction centers of PS-2 (Shutilova et al., 1982) were also first isolated by the digitonin-triton processing. The isolated preparations and developed methodological approaches to their obtainment have been widely used (Shutilova and Kutyurin, 1976; Shutilova et al., 1976, 1979, 1980, 1982, 1987, 1990, 1992, 1995; Golfeld et al., 1976; Hillier et al., 1979; Klimov et al., 1980; Scherbakova et al., 1980; Stadnichuk and Shutilova, 1980; Vershinin and Shutilova, 1980; Berthold et al., 1981; Nanba and Satoh, 1987). This has enabled the mechanisms of primary photochemical reactions to be understood more comprehensively as well as permitted the principles of the molecular organization and regularities of the functioning of the photosynthetic apparatus in plants to be ascertained.

THE MOLECULAR ORGANIZATION OF THE OXYGEN-EVOLVING COMPLEX

The Discovery of the Dimeric Structure of the OEC. The research on the thermally induced structural transitions of the OEC that was published by us in 1992 became a very important achievement in understanding the principles of its molecular organization as a dimeric complex of photosystem 2 (Shutilova et al., 1992, 1995). Based on the comparative research on the pigment-lipoprotein complexes of chloroplast membranes, the photochemical properties of the isolated photosystem 2 preparations were studied and correlated with the characteristic properties of their molecular composition as well as spectral characteristics and structure-functional role of the components entering into their structure. The isolated complex was shown to keep the high photochemical activity in the electron-transport reactions of photosystem 2. This being the case, the process of photoinduced proton release was first ascertained to take place in the structure of the PLPC PS-2, which speaks for the growth in the hydrolytic interaction of Mn cations with water under the action of light. However, the water oxidation and oxygen formation system was noted to be labile; its activity was lost in the process of isolating the PLPC PS-2 from chloroplast membranes. Its stability depended on many factors: the chemical nature of a solubilizing detergent, time of its action, solubilization mode, temperature, viscosity of a medium, its salt composition, pH-value (Shutilova, 1997 a, 2010). As a consequence, none of attempts at obtaining a photosystem 2 complex, in which the oxygen-evolving function is retained *in vitro* have been successful. The complexes that were active in oxygen evolution, i.e., the oxygen-evolving complexes (OECs) were obtained as a result of using soft non-ion detergents such as alkyl-derivatives of glucopyranose and maltose or by using digitonin or X-100 triton under the conditions of selecting the sparing membrane processing procedures during solubilization (Shutilova, 1997 a, 1999; Shutilova et al., 1990). The preparations of the isolated OEC were identical to the PLPC PS-2 complex in their pigment-protein structure, but were characterized by a twofold value of a molecular weight, i.e., they represented a dimeric association of two monomeric PLPC PS-2 (Shutilova et al., 1990).

Under these conditions, the unique property of the dimeric complex of PC-2 was discovered, namely, the complete and irreversible loss of the oxygen-evolving function during heating (33-34ºC). Using the method of differential scanning microcalorimetry, we showed that the loss of the oxygen-evolving function was accompanied by the structural transition that did not result from the denaturation of the proteins of the complex but took place due to the destruction of hydrophobic bonds and melting of saturated monogalactosyldiacylglycerides (Shutilova et al. 1992, 1995). This led to the dissociation of the dimeric OEC and formation of the monomeric PLPC PS-2 along with the concomitant thermal inactivation of the water-oxidizing center and loss of the oxygen formation function (Shutilova et al. 1992, 1995). From this, we concluded that the water oxidation and oxygen formation system arose during the hydrophobic association of the monomeric PLPC PS-2. Therefore, it was logical to suppose that the molecular structure of the water-oxidizing center was likely to be stabilized by the formation of a closed hydrophobic space (or boiler) screening the area, where the oxidative equivalents accumulated on the Mn cations bound with the reaction centers (RC) of the PLPC PS-2 in the structure of the dimeric OEC.

The question arose as to what determined the process of molecular oxygen formation in the dimeric OEC, if new components did not appear? What are the specific properties of the structure of this system that determined the efficiency of the oxygen formation reaction? The search for an answer to these questions brought us to the conclusion that the spatial association of the reaction centers of the PLPC PS-2 is likely to be such a determinant factor resulting from the formation of hydrophobic bonds.

The Hydrophobic Boiler and Its Role in the Functioning of the OEC. The analyses has showed (Shutilova, 1997 a) that the mirror-symmetric

association of the monomeric PLPCs PS-2 into the dimeric OEC results in achieving the optimally close location of the photochemical reaction centers of the monomeric PLPCs with respect to each other. This leads to stabilizing the generated photooxidized Mn cations that are formed as a result of photochemical reactions and take part in the water oxidation and oxygen formation process. It logically follows from this that the decisive role in this process is played by the structure of the hydrophobic boiler that is likely to be formed in the area, where the reaction centers of the monomeric PLPCs associate. It is the structure of the hydrophobic boiler that assigns the conditions of the screened hydrophobic environment, in which the necessary structural factors and conditions of the reaction process can be implemented. These are as follows: 1) the fixation of the spatial localization of oxidized water molecules and intermediate electron carriers with respect to each other; 2) the protection of the oxidized intermediates from the process of inactivation, and 3) the stabilization and accumulation of the oxidative equivalents of the water-oxidizing center. Only the mirror-symmetric location of the reaction centers of the monomeric PLPC PS-2 in the dimer and, primarily, the mirror-symmetric location of the hydrophobic sites of D1-proteins that are carriers of manganese cations is the condition for the formation of the structure corresponding to these requirements. This mirror-symmetric location is fixed by the structure of the hydrophobic boiler (Shutilova, 1997 a, b; 1999, 2000). Only one of four Mn cations forming part of the PLPC PS-2 was shown to take part in the photochemical process of reducing the oxidized chlorophyll $P680^+$ of the RC PS-2 and to be located in the hydrophobic area of a D1 protein, since it was not liable to extraction (Shutilova, 1997 a; 1999). In this process, the Tyr-161 amino acid residue of the D1 protein was ascertained to be an intermediate electron carrier and to play the role of a primary donor of an electron transferred to the molecule of $P680^+$. It is possible to suppose that the Tyr-161 contains a ligand OH-group connected with a benzene aromatic ring is the most preferable unidentant ligand for the coordination binding of one Mn cation taking part in its reduction (Shutilova, 1997 a; 1999). Tyr-161 was designed as a Z-donor in PS-2 that serves as a primary donor of an electron transferred to the molecule of photooxidized chlorophyll $P680^+$ in the RC PS-2 (Shutilova, 1997 a, b; 2000; Shutilova and Moiseev, 2010 a). In this connection, the functional Mn cation coordinationally bound with Tyr-161 was designated as a Z-liganded manganese cation (Shutilova, 1997 a; 1999; 2000).

All these factors enabled us to substantiate the need for the formation of the hydrophobic boiler and to build the model of the structural organization of the OEC according to the mirror symmetry rule. The research (Shutilova et al., 1992, 1995) first suggested the model of the topographic location of the major proteins of the OEC and designated the area, where the hydrophobic boiler was formed. This model is presented in Fig. 1. In all the subsequent works related to deciphering the mechanism of photosynthetic oxygen formation (Shutilova, 1997 a, b; 1999; 2000; 2010; Shutilova and Moiseev, 2010 a), this model illustrates the location of major proteins in the molecular structure of the oxygen-evolving complex of chloroplast membranes. It is important to emphasize that the suggested model is based on the symmetry in the location of the proteins during the formation of the association of two monomeric PLPCs PS-2, i.e., dimeric PLPC PS-2. The developed model was proved in the subsequent works (Zhouni et al., 2001; Ferreira et al., 2004; Loll et al., 2005) performed by the method of X-ray analysis. Thanks to the pursued biochemical and functional research and X-ray analysis, the permolecular structure of the dimeric complex of photosystem 2 was ascertained, the topography of proteins, lipids, and pigments of this complex was revealed, and the localization of its chromophore groups and co-factors of electron transport was determined. The Model of the Topographic Location of Main Proteins.

The model of the molecular organization of the OEC presented in Fig. 1 shows that this complex consists of two identical monomeric PLPC PS-2, whose major protein components are located in the structure of the dimeric OEC according to the mirror symmetry rule. The structure of each monomeric PLPC PS-2 includes 8 main proteins. They play a structure-forming and functional role. It is clear that an important structure-forming role in the monomeric complexes is played by the proteins with a molecular weight of 30 and 32 kDa designated as D1 and D2 proteins, since they are homologous to each other. These proteins determine the location of the complex in a chloroplast membrane. They fix the location of the photochemical reaction center and assign the position of chromophore groups and co-factors of transmembranous electron transport. The proteins forming the structural basis of the reaction center include cytochrome b-559 that is likely to play an important role in the process of cyclical electron transfer in PS-2. The Mn cations forming part of the complex of PS-2 are also predominately bound with the structure of D1 proteins (Shutilova, 1997 a, b; 1999; 2000; 2010; Shutilova and Moiseev, 2010 a). As we have noted above, the functionally active manganese cation is bound with the Tyr-161

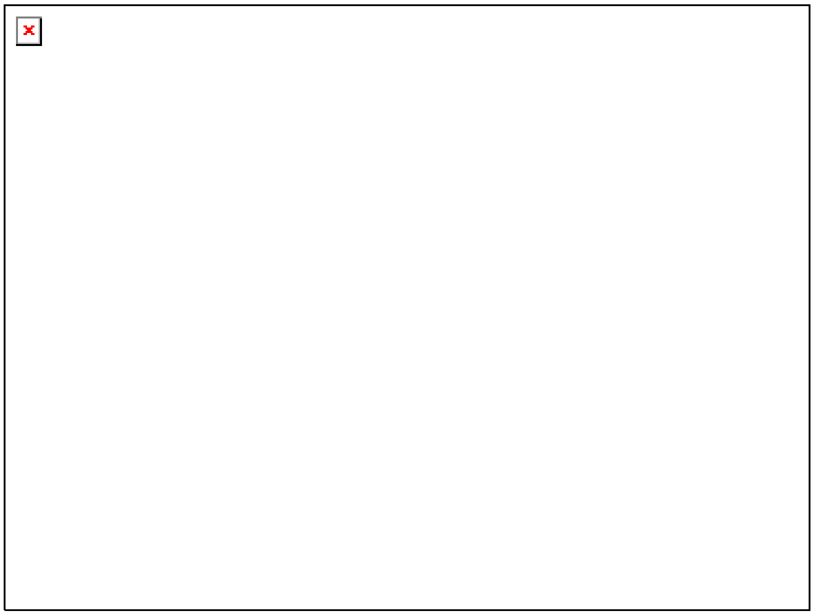


Fig. 1. Permolecular structure of the oxygen-evolving complex (OEC) of chloroplast membranes. The formation of the dimer and location of protein subunits are given. The figure shows the formation of the thermolabile hydrophobic boiler, where the unidentant Z-liganded Mn cations are located, which determine the functioning of the two-anode reactor of the water-oxidizing center of the OEC. The explanations are given in the text.

amino acid residue designated in Fig. 1 as a Z-donor. Fig. 1 shows that the RC PLPC PS-2 is surrounded with chlorophyll-proteins of the light-focusing antenna, CP43 and CP47. This being the case, it is important that CP47 contains monogalactosyldiacyl-glycerides enriched by the content of saturated fatty acids. This is likely to play an important role in the formation of the dimeric structure of the OEC (Shutilova, 1997 a; 1999). The enumerated components of the PLPC PS-2 are joined into a permolecular structure and represent the core of the PLPC PS-2. They are hydrophobic and incorporated into the structure of a thylakoid chloroplast membrane. Their biosynthesis is coded by the chloroplast genome (Vermaas, 1993; Barber, 2003).

From the lumenal side of the thylakoid, the core-complex is associated with water-soluble proteins with a molecular weight of 33, 23, and 16 kDa. These proteins stabilize the oxygen-evolving function (Shutilova et al., 1987; Shutilova, 1997 a; 1999). They are electrostatically bound with the membrane proteins and seem to form a channel for the income of water molecules to the water-oxidizing center of the complex (Shutilova, 1997 a, b; 1999; 2000; 2010; Shutilova and Moiseev, 2010 a). The biosynthesis of the water-soluble proteins in the PLPC PS-2 is coded by the genome of the nuclear DNA (Vermaas, 1993; Barber, 2003). Fig. 1 shows that a specific screen stabilizing the WC from the side of the lumen is formed by the water-soluble proteins in the area of the contact between the electron-donor sites of the reaction centers of the monomeric PLPC PS-2, where the hydrophobic boiler of the WC OEC is formed.

It is necessary to emphasize once more that the topographic location of the proteins that was suggested by us in (Shutilova, 1997 a, b; 2000; Shutilova et al., 1992, 1995) and in Fig. 1 are in a good correspondence with the data of the X-ray analysis that have also shown the symmetric location of the main proteins in the dimeric oxygen-evolving complex of PS-2 (Zhouni et al., 2001; Ferreira et al., 2004; Loll et al., 2005). Thus, since the model of the OEC suggested by us logically followed from the supposed structure of the water-oxidizing center, the circumstance that it has been proved by the X-ray analysis speak for the fact that this structure of the water-oxidizing center is correct.

The most significant element in the model suggested by us is the thesis that the Z-liganded Mn cations entering into the structure of the identical PLPCs PS-2 of the dimeric OEC have turned out to be located opposite each other in the structure of the hydrophobic boiler. This being the case, the formed conditions of the identical molecular environment enable the two-anode reactor of the water oxidation system to be formed. This will be shown below to determine the thermodynamically advantageous conditions for the synchronous oxidation of each pair of water molecules with the simultaneous O_2 formation.

It is characteristic that all the photochemical reactions, starting with the absorption of light

energy, its transformation into energy of chemical bonds with transfer of electrons and protons and ending with the process of water oxidation and molecular oxygen formation take place in the structure of the photosystem 2 dimeric complex. This complex functions as an integrated energy-transforming permolecular system, and the processes taking place in this system cannot be structurally disconnected. In this respect, this complex is similar to another permolecular complex of chloroplast membranes, namely, the ATP-synthase complex (Riznichenko et al., 2009). Oxygen is a final product of the functioning of the photosystem 2 dimeric complex. Therefore, in compliance with the rules of biochemical terminology, this dimeric complex must be called according to the final product of the reaction, i.e., simply "an oxygen-evolving complex" (Shutilova, 1997 a, b; 1999; 2000; 2010; Shutilova and Moiseev, 2010 a).

MECHANISM OF PHOTOSYNTHETIC WATER OXIDATION AND MOLECULAR OXYGEN FORMATION

The Four-Quantum Cycle of Accumulation of the Oxidative Equivalents of the Water-Oxidizing Center in the OEC. Earlier, we have analyzed the stages of the molecular photochemical processes of the formation of the oxidative equivalents, their accumulation, and formation of the water-oxidizing center in the hydrophobic boiler of the dimeric OEC (Shutilova, 1997 a, b; 1999; 2000; 2010; Shutilova and Moiseev, 2010 a). The scheme joining these processes is given in Fig. 2. Since the formation of each O_2 molecule requires the absorption of four light quanta, it is necessary that the processes of the two-quantum reduction of the final electron acceptor plastoquinone Q_B (PQ_B) and four-quantum accumulation of the oxidative equivalents in the water oxidation system of the complex be matched. When a light quantum is absorbed in turn in the left and right PLPC PS-2, the primary reaction of removing an electron from the photoexcited chlorophyll P680* that serves as a trap for light energy takes place in the structure of the left and right PLPC PS-2. Then, the electron is transmembranously transferred to pheophytin, plastoquinone Q_A, non-heme iron, and plastoquinone Q_B located on the acceptor side of the OEC that is exposed to the thylakoid stroma. The plastoquinone Q_B can join only two electrons; therefore, the formation of PQ_B^{2-} transfers the PLPC PS-2 into the closed state. Then, PQ_B^{2-} diffuses into the membrane and must be replaced by new molecules of the oxidized PQ_B. For this time, the photochemical reactions take place in the second PLPC PS-2 of the dimeric OEC, which determines the possibility of using two more light quanta to transfer the next two electrons to the PQ_B acceptor of the second PLPC PS-2. The electron vacancy on the chlorophyll of the RC ($P680^+$) that is formed in the process of the photochemical reaction in the monomeric PLPC PS-2 is filled by the Tyr-161 amino acid residue (a Z-donor), which is in its turn reduced from Mn^{2+} cation coordinationally bound Tyr-161 (Fig. 2). The four-quantum cycle results in removing two electrons from each of the Z-liganded Mn^{2+} cations of the left and right PLPC PS-2. Consequently, four oxidative equivalents or four electron vacancies are accumulated on two oppositely located Z-liganded Mn^{4+} cations in the process of the cooperative functioning of the reaction centers in the dimeric OEC. This is how the two-anode reactor of the water oxidation system is formed, in which the subsequent synchronous oxidation of two water molecules and oxygen formation take place. The steady electron transfer to the system of transmembanous electron transport and multiple repetition of the four-quantum cycle are ensured thanks to the matched functioning of two reaction centers in the OEC. According to the suggested concept, the two-anode reactor is the basis of the water-oxidizing center in the OEC of higher plants. Its stabilization is implemented thanks to the structure of the hydrophobic boiler that protects the reaction space of the water-oxidizing center from the impact of the environment (Shutilova, 1997 a; 1999). The suggested structure of this water-oxidizing center determines an exclusive advantage that the oxidative potential is photogenerated on the identical Z-liganded Mn cations of the dimeric OEC. This potential is assigned by the same degree of oxidation of these manganese cations and identity of their molecular environment owing to that fact that each of them enters into the structure of the identical and symmetrically located RC PLPC PS-2 in the composition of the hydrophobic boiler of the dimeric OEC (Fig. 1, 2). Therefore, the symmetric two-anode reactor is formed that makes it possible to implement the equal-rate but oppositely directed electron outflow from the oxidized substrate (in our case, it is a water molecule). The entrance of Mn cations into the structure of the OEC is likely to be explained by the presence of vacant orbits in the electron shell of a Mn atom, high coordination number, and alternating ion radius that depends on the value of charge of an atom nucleus (Shutilova, 2006; Shutilova and Moiseev, 2010 b). This determines the participation of Mn cations in multi-electron processes in chemistry and biology (Semenov et al., 1975). The suggested construction of the WC corresponds to the experimental data speaking for the lability of its structure. It is also possible to suppose that the possibility of dissociation of the dimeric OEC is a factor regulating its functional activity.

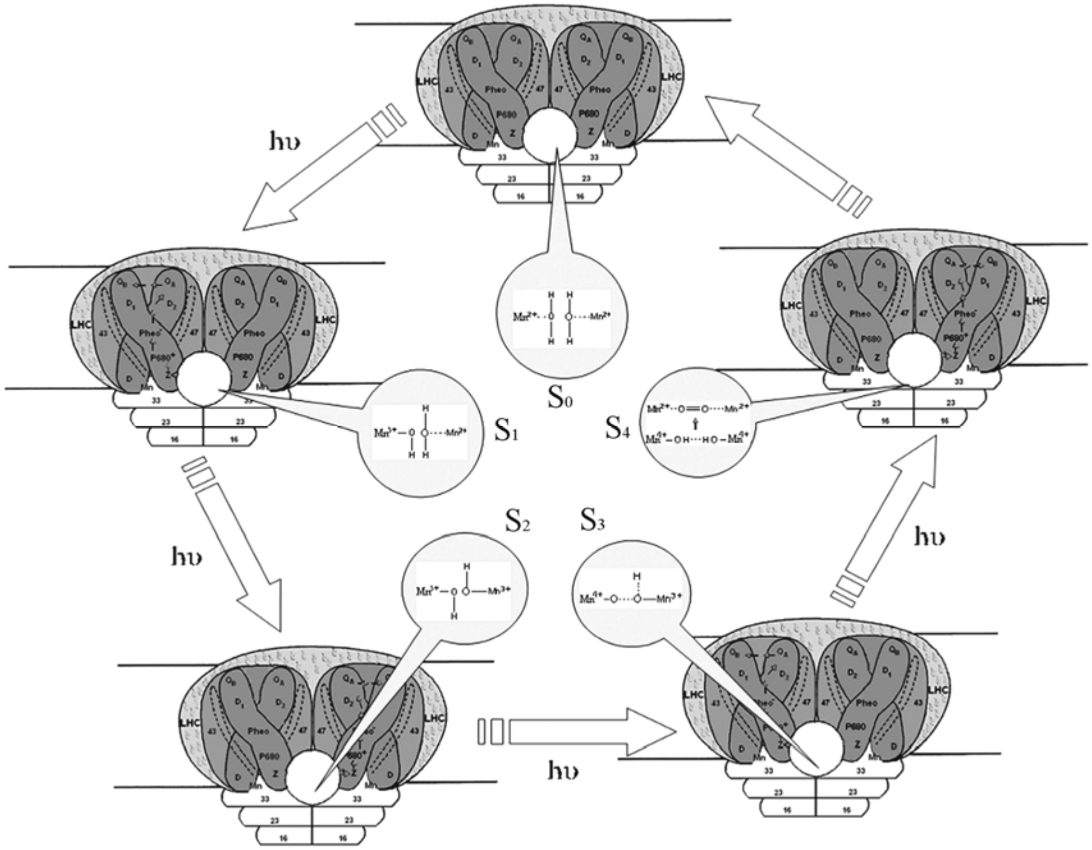


Fig. 2. Scheme of the process of water molecule oxidation and oxygen formation taking place in the structure of the hydrophobic boiler of the oxygen-evolving complex (OEC) of chloroplast membranes for the period of the four-quanta cycle of photochemical reactions. Arrows show absorption of light quanta and transition of the complex into the S_i oxidized state as a result of the photochemical reaction and electron transfer in monomeric PLPCs PS-2 that make up the structure of dimeric OEC. The center of the figure reflects the process of accumulation of oxidative equivalents in the hydrophobic boiler of the water-oxidizing center of OEC and the reaction of synchronous four-electron water molecule oxidation and O_2 formation.

The Theoretical Analysis of the Factors of Synchronous Water Molecule Oxidation and Oxygen Formation. According to the theory of molecular orbitals, the formation of a sigma- and pi-bond between oxygen atoms of oxidized water molecules is determined by their "head-on" interaction, since a sigma-bond has an axis of cylindrical symmetry and can arise in this case only under the condition of synchronous and equal-rate electron outflow from the oxidized hydroxyl groups of water molecules in opposite directions along the coordinate line of the reaction. This thesis has been confirmed by the quantum-chemical analysis (Shutilova, 2010; Shutilova and Moiseev, 2010 a, b). The very important conditions for molecular oxygen formation in this reaction are that the oxidative potential of the oxidizer molecules, toward which electrons flow from water molecules, must be equal and sufficient. This requirement is met by the suggested construction of the WC in the dimeric OEC, where these conditions are implemented. The two-anode reactor of the WC in the dimeric OEC arises as a result of oxidizing the left and right Z-liganded manganese cations to the valent state Mn^{4+} in the process of the photochemical reactions taking place in the structures of the RC PLPC PS-2 (Fig. 2). One can see that the formed two-anode reactor promotes the synchronous detachment of two electrons from each hydroxyl group of two water molecules and outflow of each pair of electrons in opposite directions toward the photochemically oxidized manganese cations that are spatially located opposite to each other in the structure of the hydrophobic boiler of the dimeric OEC (Fig. 2). The electron vacancies on oxygen atoms arising in this process are compensated for at the expense of the formation of a sigma- and pi-bond between oxygen atoms. The formation of the hydrophobic environment of the water-oxidizing center in the boiler of the OEC determines its stability and efficiency of the O_2 formation reaction.

The Reactionary Process of Water Oxidation and Molecular Oxygen Formation. Let us consider the stages of proceeding reactions in detail. It is shown (Fig. 2) that the first stage of the process is the inclusion of hydroxyl anions of water molecules into the coordination sphere of the Z-liganded Mn^{2+} cations during their photooxidation to Mn^{3+}. This results from the hydrolytic interaction of formed Mn^{3+} cations with water and is considered as a stage of photohydrolysis (Shutilova, 1997 a, b; 2000; Shutilova and Moiseev, 2010 a). Fig. 2 shows that the photooxidation of the Z-liganded Mn^{2+} cations in the left and right RC of the dimeric OEC that takes place during the absorption of the first two light quanta results in the formation of two oppositely located hydroxides Mn^{3+}(OH) and release of the first two protons. As a result of photohydrolysis, the manganese cations become the carriers of the hydroxyl groups of oxidized water molecules, which promotes the stabilization of the oxidized manganese cations and necessary spatial orientation of hydroxyl anions of water molecules with respect to each other. Oxygen formation requires the approachment of two hydroxyl anions to the distance of the Van Der Waals interaction (Shutilova, 1997 a). The Ca cations that make a structuring impact and promote the formation of the two-nuclear dihydroxide association [Mn^{3+}(OH)···(OH)Mn^{3+}] were shown to play the key role in this process. As a consequence of this, the concentration of Ca^{2+} in the medium can serve as a regulator of the photosynthetic activity of the OEC (Shutilova et al., 1987). After the third and fourth quanta are absorbed, the further photochemical oxidation of Mn^{3+} cations takes place in the left and right PLPC PS-2, and two Mn^{4+} cations are formed. This results in the formation of an unstable two-nuclear association [Mn^{4+}(OH)···(OH)Mn^{4+}], in which electron density disproportionation between the manganese cations and oxygen atoms takes place (Shutilova, 1997 a, b; 1999, 2000, 2010; Shutilova and Moiseev, 2010 a). This is caused by the high oxidative potential of the Mn^{4+} cations and oppositely directed electron outflow from the OH-groups bound with the manganese cations. Such conditions determine the rate and efficiency of the spontaneous reaction of electron density disproportionation in this system with the formation of a bond between oxygen atoms of hydroxyl anions, which was described in detail in (Shutilova, 1997 a). Meanwhile, as Fig. 2 shows, two electrons are transferred in opposite directions from one hydroxyl group to the Mn^{4+} cation of the left PLPC PS-2 and, correspondingly, from another hydroxyl group to the Mn^{4+} cation of the right PLPC PS-2. The manganese cations are reduced in this process to Mn^{2+}. The formation of an electron vacancy on the hydroxyl groups results in the formation of electronic orbitals between their oxygen atoms. This process is accompanied with the energetically advantageous formation of free molecular oxygen that evolves in the atmosphere. As a result, the four-electron oxidation of the hydroxyl anions of two water molecules (embedded into the coordination sphere of the Z-liganded manganese cations) is performed, molecular oxygen evolves, protons are released, and the water-oxidizing center returns to the initial state that is necessary for the new cycle of the reactions of water molecule oxidation and O_2 formation. The scheme given in Fig. 2 shows the transitional oxidative states of the water-oxidizing center to correspond to the S-states of the four-quantum cycle of oxygen molecule formation.

QUANTUM-CHEMICAL MODELING

The developed mechanism has been proved by the quantum-chemical analysis. The analysis has been carried out using the Priroda-6 software complex by the DFT approach of the PBE density functional with the SBK basis set (Laikov, 1997). The method enables the distribution of the valent electron density to be calculated by estimating the total energy of interaction between molecules in the system under consideration, permits one to analyze the surface of the potential energy of the system and to optimize its geometry. The distance (4.03 Å) between Mn^{4+} cations and orientation of manganese atoms in the dihydroxide association [Mn^{4+}(OH)···(HO)Mn^{4+}] were the initial parameters. Figure 3 shows the energy profile of the O_2 formation reaction. It schematically reflects the distribution of electron density for HOMO- and LUMO molecular orbitals during the disruption of Mn-O bonds and shows the dynamics of the formation of an O-O bond as a result of electron transfer to Mn^{4+} cations (Fig. 3). The graph in Fig. 4 reflects the course of change in the distance between the manganese cations and oxygen atoms in the reactionary process of reduction of Mn^{4+} to Mn^{2+}. Fig. 5 shows the simultaneous process of the formation of an inter-atomic bond between oxygen atoms of the oxidized hydroxyl groups in the dihydroxide association under consideration. The formed oxygen evolves in the environment as a gaseous product, which makes the process of photosynthetic water oxidation highly effective and irreversible.

The origin of the molecular structure of the dimeric OEC should be regarded as a paramount event of biological evolution on our planet that determines the development of life on the Earth. It is necessary to emphasize that the permolecular structure of the oxygen-evolving complex is a unique

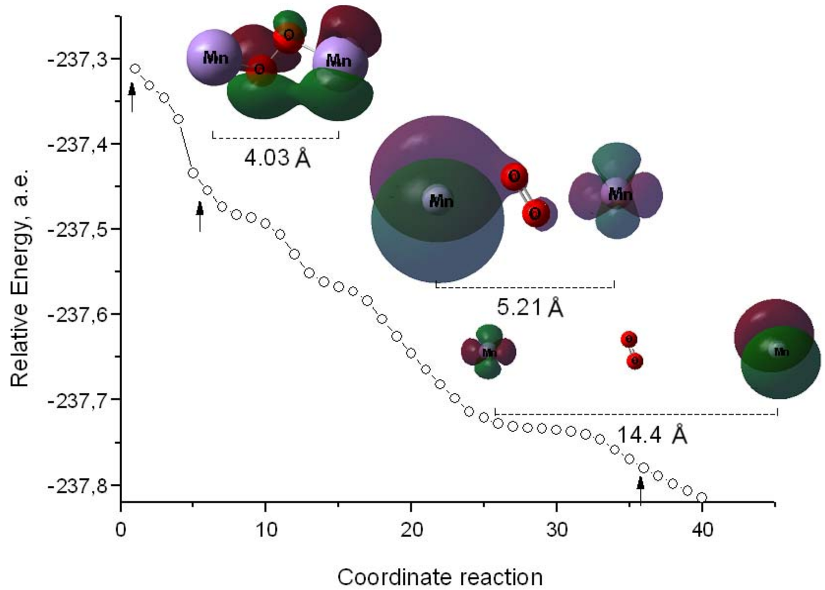


Fig. 3. Quantum-chemical modeling of the electron density disproportionation and oxygen formation reaction in binuclear manganese dihydroxide associate [Mn(OH)···(HO)Mn] which is formed according to the developed mechanism in the course of the four-quanta cycle in the structure of the water-oxidizing center of the OEC. The figure shows the energetic profile of the electron density redistribution process in this system, which is accompanied by O_2 formation and reduction of manganese cations. The graph reflects the change in the distance between Mn^{4+} cations (4.03 Å) at the beginning of the reaction and distance between Mn^{2+} cations (14.4 Å) at the end of the process.

and only structure in nature, in which the oxygen formation function is performed. It is clear that this process is implemented thanks to the construction of the molecular two-anode reactor of the photosystem 2 water-oxidizing center, and, as the thermodynamic analysis shows, it does not seem possible to imagine the alternative solution of this problem. Meanwhile, there is a concept about the role of the μ-oxi-bridge structural complex in the formation of molecular oxygen (Vrettos et al., 2001; Umena et al., 2011). These ideas are incompatible with the data on the isotopic composition of photosynthetic oxygen that prove the direct oxidation of water molecules and not any other oxygen-containing compound (Kutyurin, 1970). In addition, the rate of the isotopic exchange of oxygen atoms in μ-oxi-groups of the metal-organic artificial complexes is five orders of magnitude lower than in the water-oxidation system of plant chloroplasts (Hillier and Wydrzynski, 2001).

The formation of the molecular two-anode reactor of the WC took place in the process of evolution of plant organisms and is evident to originally occur in the structure of the monomeric PLPC PS-2. This is evidenced by the molecular organization of the monomeric PLPC PS-2. This organization is determined by the structure of the homologous D1 and D2 proteins that are identical to each other in the area of the hydrophobic nucleus embedded into a membrane and contains a bound manganese (Shutilova, 1999, 2010). The monomeric PLPCs PS-2 of higher plants are characterized by a high photochemical activity of the reaction center as well as by the processes of photoinduced electron transport and proton release taking place in them (Shutilova, 1980). This being the case, the oxygen-evolving activity of the isolated monomeric complex is not retained owing to the structure of the hydrophobic boiler of the WC being destroyed by solubilizing detergents in the process of preparative isolation of the complex. However, according to the data of (Takahashi et al., 2009), evolutionally more ancient organisms such as acidophilic and thermophilic red algae and cyanobacteria are characterized by the predominant content of the monomeric complexes that are active in oxygen evolving. This is evident to be determined by their aquatic habitat and rigid membrane structure that is typical for these organisms. Higher plants, in which lipids are dominant in the structure of thylakoid membranes enriched with the content of non-saturated fatty acids, are characterized by a high fluidity of membranes and possibility of association of the monomeric PLPC PS-2 into a dimer. Thanks to these factors, the conditions of the predominant functioning of the dimeric OEC could arise in the evolutionary process, since the potential for using

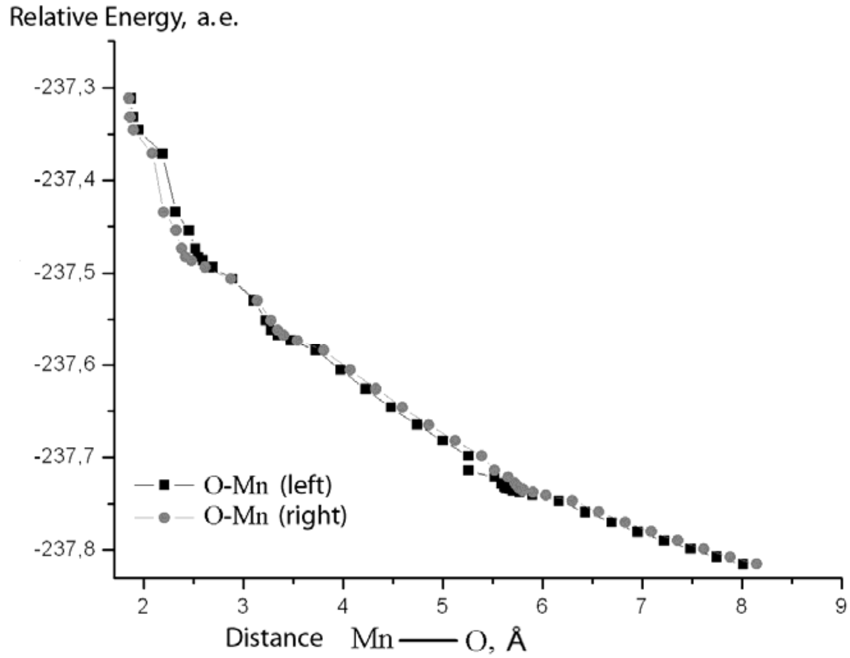


Fig. 4. Dynamics of change in the distance between manganese cations and oxygen atoms in the course of the process of oxidation of the liganded hydroxyl groups in the dihydroxide associate in the structure of the water-oxidizing center of the OEC and reduction of cations Mn^{4+} to Mn^{2+} in the monomeric complex of the left and right PLPCs PS-2.

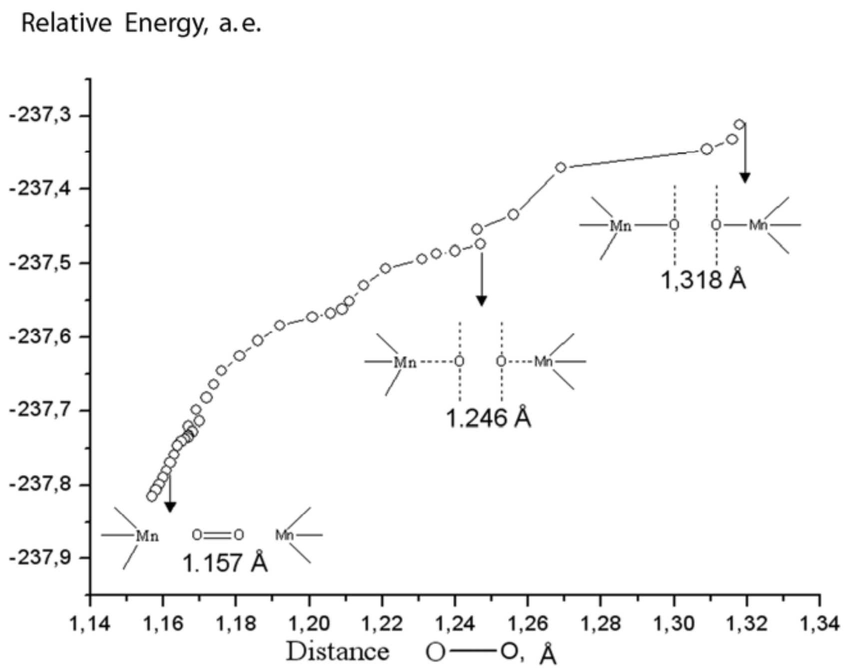


Fig. 5. Change in the distance between oxygen atoms in the course of the reaction of electron density disproportionation in the binuclear manganese dihydroxide associate [Mn(OH)···(HO)Mn]: from 1.318 Å in the initial associate to 1.157 Å in an oxygen molecule.

absorbed light is doubled in such a complex. One more advantage is that oxygen formation can undergo a finer regulation of matching with the processes of transmembranous electron transport depending on the need of a plant.

The organization of the manganese cluster of the oxygen-evolving complex in chloroplast membranes was considered in detail in the research by (Shutilova, 1997, 1999). Based on the biochemical research, the four-nuclear manganese cluster of the monomeric core-complex was shown to have a heterogeneous structure: three manganese atoms are localized in the hydrophilic loop of the D1-protein of the RC and are easily extracted with salt solutions, urea, concentrated buffer, and one atom is fixedly bound with the structure of the hydrophobic C-spiral site of the D1-protein penetrating a membrane. The manganese cation included into the hydrophobic area was substantiated to be functional in the process of water molecule oxidation owing to the formation of a coordination bond with the unidentant ligand Tyr-161 that plays the role of an intermediate electron carrier to the molecule of photooxidized $P680^{+}$ from the Tyr_{Z}– liganded Mn cation. This promotes the stable localization of the electron vacancy on the coordinationally bound manganese cation with the formation of Mn^{3+} during the absorption of the first light quantum and with the formation of Mn^{4+} during the absorption of the next light quantum (Fig. 2). There are two manganese cations of this type in the structure of the dimeric OEC; they are located symmetrically, and the photochemical oxidation of each in the water oxidation system results in the absorption of four light quanta and in the formation of the two-anode reactor. The two-anode reactor can also be formed as a result of the four-quantum succession of photochemical reactions in the structure of the monomeric complex of PS-2, where the role of the second functional manganese cation is played by the Tyr_{D}-liganded manganese cation.

The data on the rontgenography of the structure of the oxygen-evolving complex of cyanobacteria in the research by (Zouni et al., 2001, Ferreira et al., 2004, Loll et al., 2005, Takahashi et al., 2009, Umena et al., 2011) proved the topography of protein subunits in both the dimeric and monomeric complex of PS-2 that had been suggested by us based on the biochemical data (Shutilova et al., 1992, 1995, 1997, 1999). However, as for the molecular organization of the water oxidation system, the methodology of X-ray analysis and, in particular, technology for preparing crystals, irradiating with X-rays, and obtaining rontgenograms do not enable the functional state of the WC OEC to be conserved. In addition, X-ray analysis is restricted by its being static and not permitting different short-living structural states to be reproduced on its basis. The interpretation of electron density maps used by the authors when obtaining rontgenograms with a resolution of 1.9Å (Umena et al., 2011) enables diffractional rontgenograms to be considered from the standpoint of the location of separate groups of metal atoms. However, it is possible to suppose that the manganese cations of the native WC gather during the used procedures into the manganese cluster, whose functional role is incomprehensible from the standpoint of the chemical process of water oxidation and molecular oxygen formation. The authors assert it to be possible that oxygen atoms are located pairwise as oxi-bridge structures (oxygen bridges) in the four-nucleus manganese cluster. The mechanism of water molecule oxidation including the stage of the concert detachment of an electron and proton from a water molecule with the help of the Tyr-161 radical formed during the photochemical excitation of the RC PS-2 was suggested earlier (Hoganson and Babcock, 1997). In the opinion of these authors, the reaction can take place as a result of the conjugation of this process with the dissociation of the proton in the hydroxyl group of the Tyr-161 molecule owing to the influence of its aromatic nucleus and possibility of proton transfer through the tautomeric transition in the neighboring molecule of histidine (His-190). The model was called the "Concerted hydrogen-atom abstraction in photosynthetic water oxidation". According to the suggested model, oxygen formation results from stabilizing first one (S_1) hydroxyl radical and then the second (S_2) hydroxyl radical on the manganese cluster. However, the research based on the model systems showed the rate of water molecule oxidation in the structure of such clusters to be five orders of magnitude lower than in chloroplasts during photosynthesis. In addition, as has been discussed in the beginning, hydroxyl radicals are also unacceptable to be considered as intermediate products of the successive single-electron water molecule oxidation during photosynthesis from the standpoint of their destructive effect on biological structures. This makes it impossible to use this model as an analogy in order to interpret the process of photosynthetic molecular oxygen formation.

In contrast to the cited work, the scheme of molecular oxygen formation presented in Fig. 2 is based on the theoretical substantiation for the chemical process of formation of an interatomic bond in an oxygen molecule and shows the need for the synchronous pairwise water molecule oxidation in the structure of the two-anode reactor of the WV

OEC. The synchronous oxidation of hydroxyl water molecule anions is shown to take place only in the S_4 oxidized state. The scheme presented in Fig. 2 shows that the hydroxyl water molecule anions included into the dihydroxide association are also characterized by the location of two oxygen atoms at the distance of the Van der Waals interaction regulated by calcium cations. Possibly, it is the location of oxygen atoms that must be at issue when interpreting electron density maps. Apparently, this can be shown by the subsequent more exact research by the method of nuclear magnetic resonance.

It is undoubted that the suggested mechanism of molecular oxygen formation is a strictly four-quantum process despite the fact that only two functional Tyr_Z-liganded manganese cations symmetrically located in the structure of the D1 proteins of the dimeric OEC are stated by the suggested concept to take part in the reaction. According to the second law of photochemistry, the absorption of one photon (or light quantum) can result in the detachment and transfer of only one electron from a donor molecule to an acceptor molecule. Consequently, the transfer of four electrons from two initial Mn^{2+} cations requires the absorption of four light quanta and results in the formation of two Mn^{4+} cations. The scheme of Fig. 2 designates each new oxidized state of the WC as an S-state according to the accepted terminology (Kok et al., 1970). The parallel symmetric location and possibility of the simultaneous excitation of the left and right RC in the dimeric OEC must not cause any embarrassment, since this does not change the essence of the process – each photochemical reaction of electron transfer in each PLPC requires the absorption of its own photon. The spatially close location of photooxidized functional manganese cations and identity of their molecular environment are very important. This is what explains the uniqueness of the organization of the water-oxidizing center that determines its capability to form molecular oxygen from the most heavily oxidized but widely accessible substrate (water molecules) by the synchronous two-anode oxidation of each pair of water molecules according to the mechanism of the reaction process suggested in this work. The efficiency of this process has been proved by the method of quantum chemical modeling.

We can conclude that the suggested mechanism of formation of free oxygen by the two-anode synchronous pairwise oxidation of two water molecules in each four-quantum cycle of photochemical reactions in the OEC of plant chloroplasts has a universal character and can be regarded as a key process of the use of solar light energy for the development of life on the Earth. The suggested mechanism of the two-anode synchronous oxidation of water molecules with oxygen formation can be used to comprehensively decipher this process in nature, to model it in nanotechnology, and to create the systems of artificial photosynthesis.

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